 ISSN 2278-1587(Online)

GENETIC PROFILING OF SPIDERS OF SEMI-ARID HABITAT BY USING DEHYDROGENASE AND ESTERASE ISOZYME

Anjali and Sant Prakash*

Molecular Genetics lab, Dept of Zoology, Dayalbagh Educational Institute, Agra-282005, India

*Corresponding author- Sant Prakash

santprakashdr@gmail.com

ABSTRACT

The activity and expression levels of alcohol dehydrogenase, glucose-6-phosphate dehydrogenase and non-specific soluble esterase were compared in the seven species (*C. lyoni, Hersilia sp. Oxyopes sp., Menemerus bivittatus, Leucauge decorata, Selenops sp. and Cyrtophora citricola*) from the semi-arid habitat of Agra region. Three predominant non-specific esterases loci (Est1, Est2 and Est3) were recorded in different spiders. ADH, G6PD and non-specific esterases profiles of spiders showed close confirmation to the invertebrate fauna. The significant correlation between the Isozyme activity was established in the form of evolutionary models for the above seven species.

Key words- Isozymes, ADH, G6PD, non specific soluble esterase, spiders, semi-arid area, Agra.

INTRODUCTION

Isozymes are the multiple enzyme forms of enzymes. Their studies are important not only for determining inter-specific relationships but also in revealing genetically controlled variants among populations. Such genetic variants provide markers for the investigations of the intra and inter relationships among populations. Substitution in amino acid leads to differences in enzymes in electrophoretic mobility at different rate. This change in mobility occurs because enzymes of the same size and shape move at a rate determined largely by the ratio of the number of positively charged amino acids to the number of negatively charged ones. Special procedure is usually required to detect genetic variations because most of it is hidden variations not apparent at phenotypic level. So it became important to determine how genetic variation is organized into genotypes. These events are probably closely associated with morphological, physiological, biochemical ontogenetic alterations. The changes in isozyme pattern may be tissue or cell specific and indicate regulatory event at genetic an epigenetic levels for providing genetic marker for species identification and habitat influence on insect population.

Agra is the region which falls under the semi-arid habitat and maximally covered by the Yamuna River (Anjali and Prakash 2012).The different agro-ecosystems in this region support the growth and development of spiders and spiderlings. The cytogenetical database on the predominant spiders of this region was provided by Anjali and Prakash 2014. This study is another continuation of the targeted predominant spider species for the isozyme profiling.

Three isozymes are focused in the present study (Alcohol dehydrogenase [ADH] Glucose -6- phosphate dehydrogenase [G6PD] and Esterases). Alcohol dehydrogenase [ADH, E.C. 1.1.1.1] is NAD+ dependent enzyme that catalyzes the reversible interconversion of a huge number of alcohols and their corresponding aldehydes and ketons. This enzyme is cytoplasmic and exhibits broad substrate specificity (Edenberg, 2007). The second enzyme focused was G6PD (Glucose -6-phosphate dehydrogenase, E.C. 1.1.1.49). Last enzyme used was soluble esterases which belong to the group

of hydrolytic enzymes that splits the esters into acid and alcohol in chemical reactions, ubiquitous in nature but their exact role in the different physiological functions is not known (Park, 1999). The records on spiders are limited, and the present work provides basic information of genetical profiling and expression of isozyme in the different spider species.

MATERIALS AND METHODOLOGY

The activity and expression levels of Alcohol dehydrogenase, Glucose-6-phosphate dehydrogenase and non-specific soluble esterase were estimated in seven spider species, *C. lyoni, Hersilia sp. Oxyopes sp., Menemerus bivittatus, Leucauge decorata, Selenops sp. and Cyrtophora citricola.* The spiders were collected from the semi-arid habitat of Agra region by hand picking.

Homogenization: - Spider legs were removed and body was homogenized in extraction buffer 0.25M sucrose (1:10) using hand hold glass homogenizer and kept in cold condition.

Enzyme extraction – Homogenate was centrifuged at 12,000 rpm for 10 mins at 4ºC. The resultant clear supernatant was refrigerated at 4ºC.

Quantification of protein: - Extracted proteins were quantified by using spectrophotomer at 660 nm wavelength.

Electrophoresis: - The electrophoresis technique was followed as given by Sambrook *et al.,* (2000).

Staning was according to Prakash (1990) and gels were stored at 37ºC for 20 to 40 minutes in dark. Then the stain was removed and gels were kept in distilled water and photographed.

RESULTS

Concentration of proteins

The concentration of proteins in different spider species was recorded in which the *C. lyoni, Phidippus* sp.and *Leucauge decorata* showed the maximum concentration at 660 nm (Fig 1).

ADH is a cytoplasmic enzyme and exhibits broad substrate specificity. The ADH bands obtained through PAGE by using ethanol as a substrate. ADH is a dimer A and B and combinations of these resulted in heterodimer AB. All species exhibited B and AB heterodimer. ADH-A band was observed in *Menemerus bivittatus* and *Selenops* sp. Variations were observed in the intensity of the B locus in different species, prominent intense band was observed in *C. lyoni, Cyrtophora citricola* and *Leucauge decorata* (Fig 2).

Glucose 6-phosphate dehydrogenase was subjected to electrophoretic studies in order to standardize its species specific expression. A maximum of two G6PD phenotypes were resolved from two species (*C. lyoni* and *Cyrtophora* sp.). G6PD-B was present in both the species in the cathodal zone. G6PD-A in C. lyoni was marked with characteristic broad band width. Since G6PD locus is X-linked, females of spiders exhibited both the bands G6PD-A and G6PD-B, which confirms its sex differential pattern (Fig 3).

Soluble nonspecific esterase of animals provide significant information for the understanding of biochemical and genetical process in an organism, as they were among the first enzyme to be studied by the combined technique of zone electrophoresis and histochemical staining. Two characteristics set the enzyme apart, one that there is a large number of esterase gene loci in various groups of species and second that is also overlapping of substrate specificity.Three major functional zones of non-specific esterase activity have been detected in the adults of *Crossopiza lyoni , Menemerus* sp*., Cyrtophora citricola, Hersilia* sp., *Leucauge decorata* and *Selenops* sp. EST-1 is higly anodal while EST-3 is cathodal (Fig 4). EST-1 is most active zone as designated in decending order followed by Est3, Est 2.Est1.

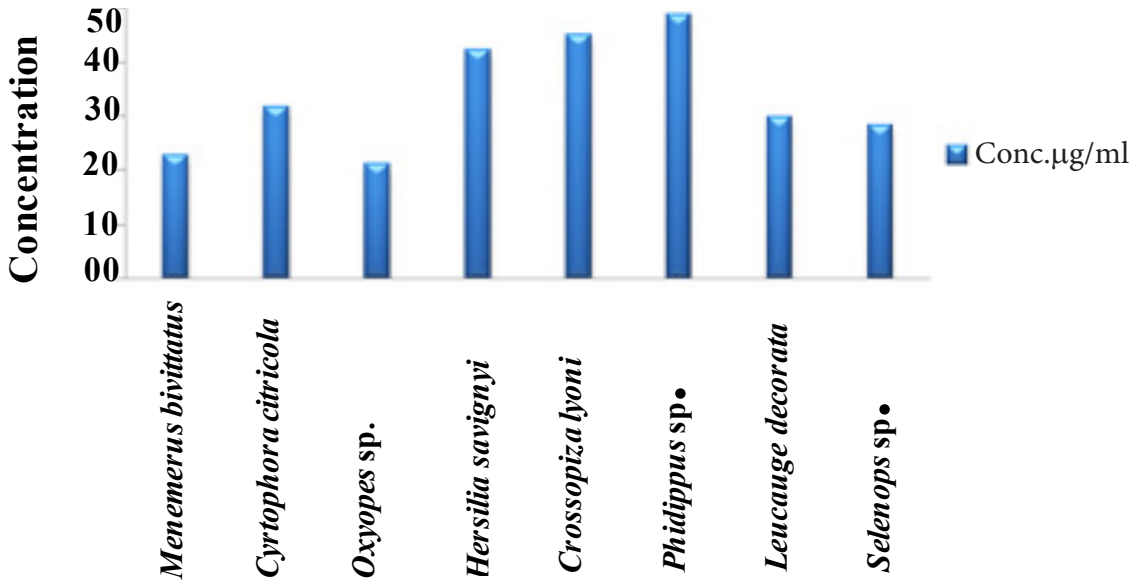


Figure - 1, Concentration of proteins ug/ml in spider species

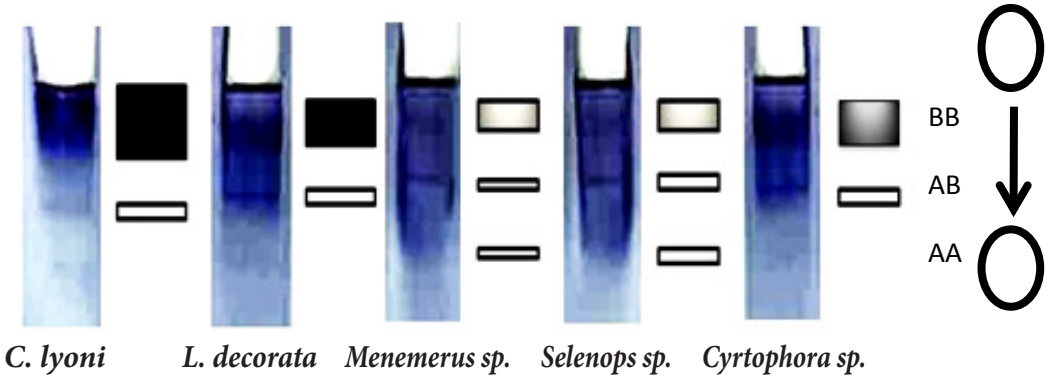


Figure - 2, Comparative ADH profile for Spiders with Zymogram

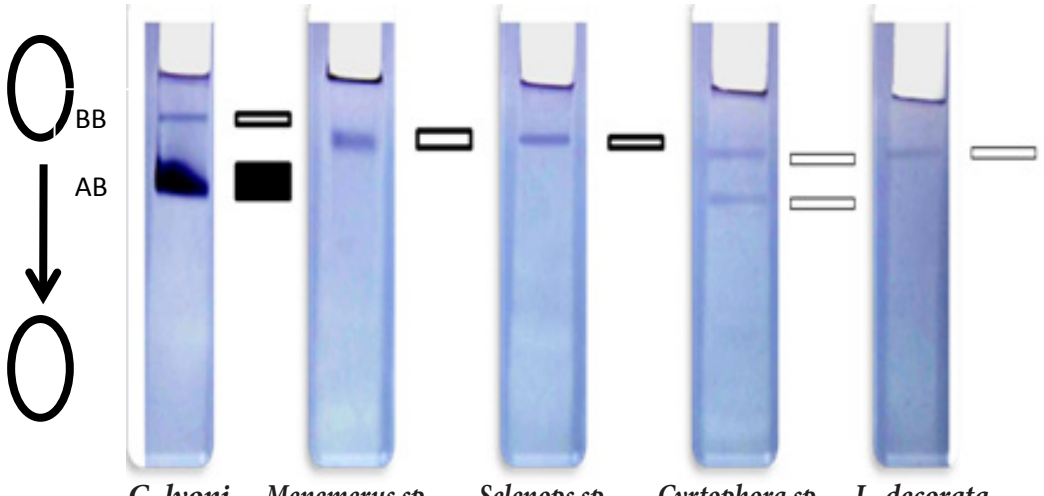
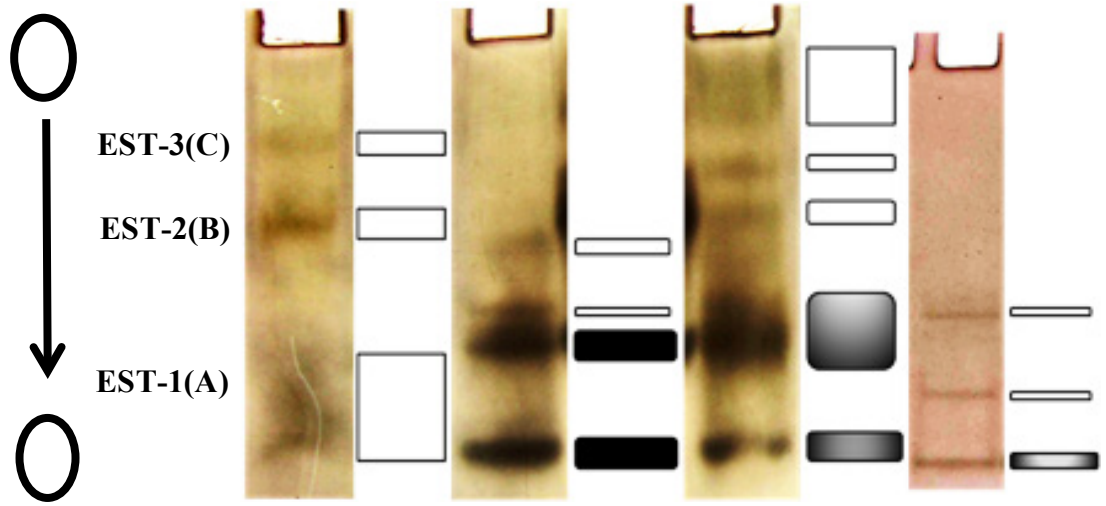
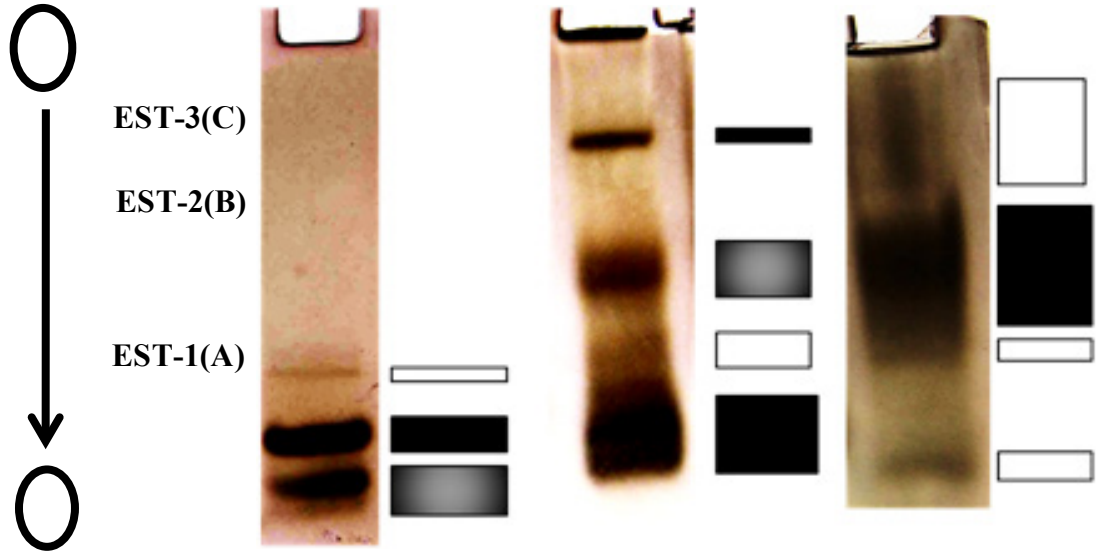


Figure - 3, Comparative G6PD profile for spiders with Zymogram



1.C. lyoni 2.Menemerus sp. 3.Cyrtophora sp. 4.L. decorata



5. Selenops sp. 6. Oxyopes sp. 7 . Hersilia sp.

Figure - 4, Comparative Esterase profile for spiders with Zymogram

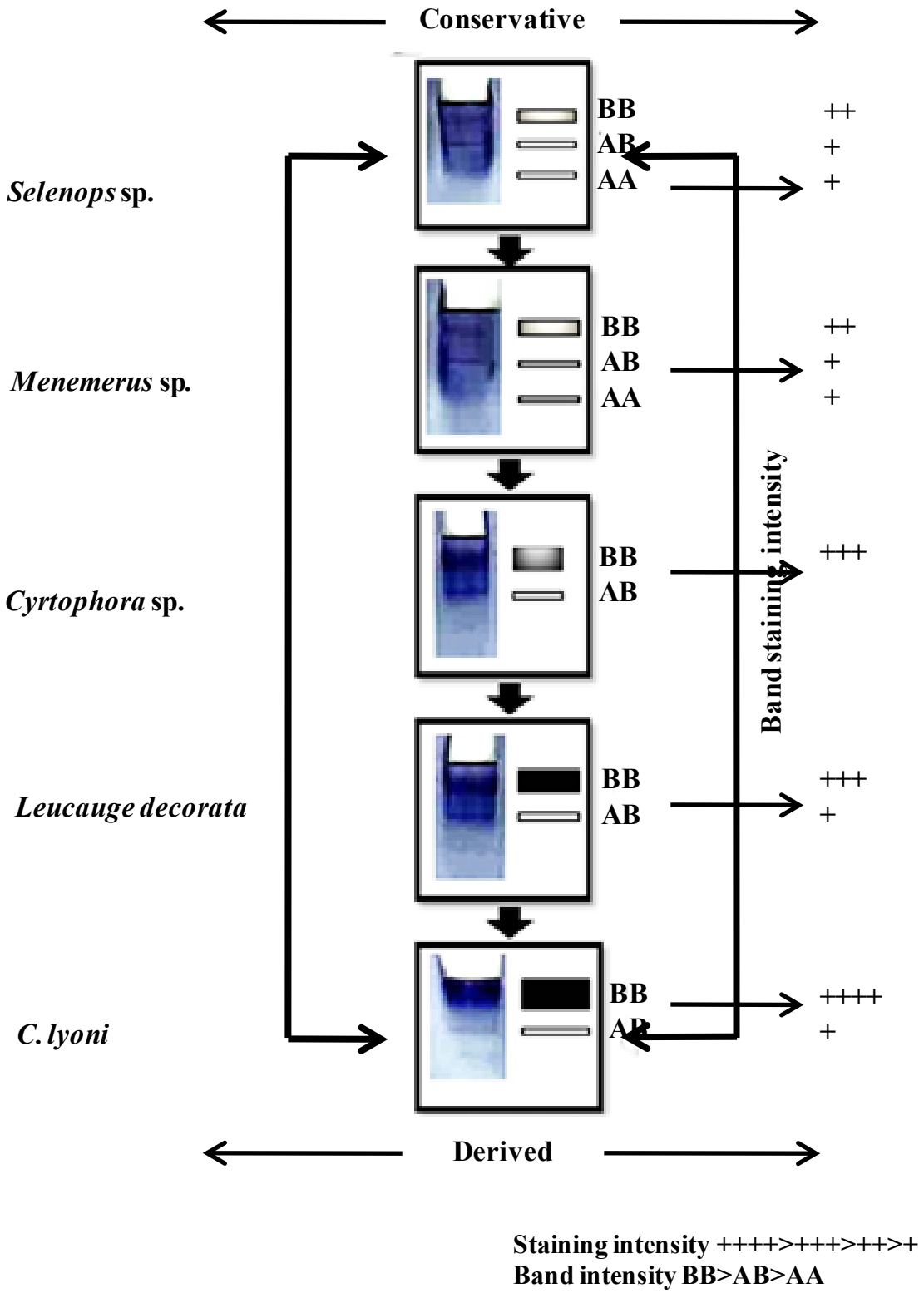


Figure - 5, ADH model for spiders

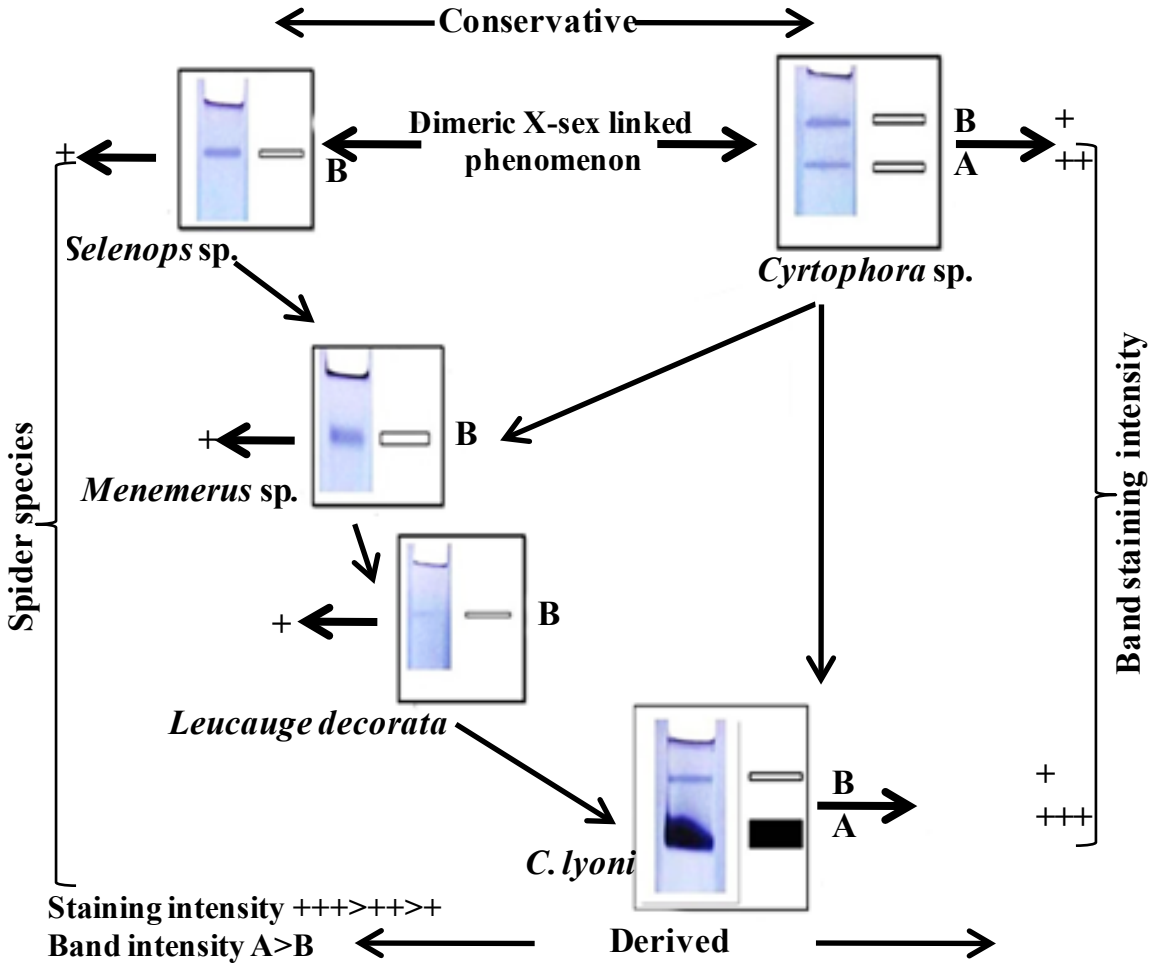


Figure - 6, G6PD model for spiders

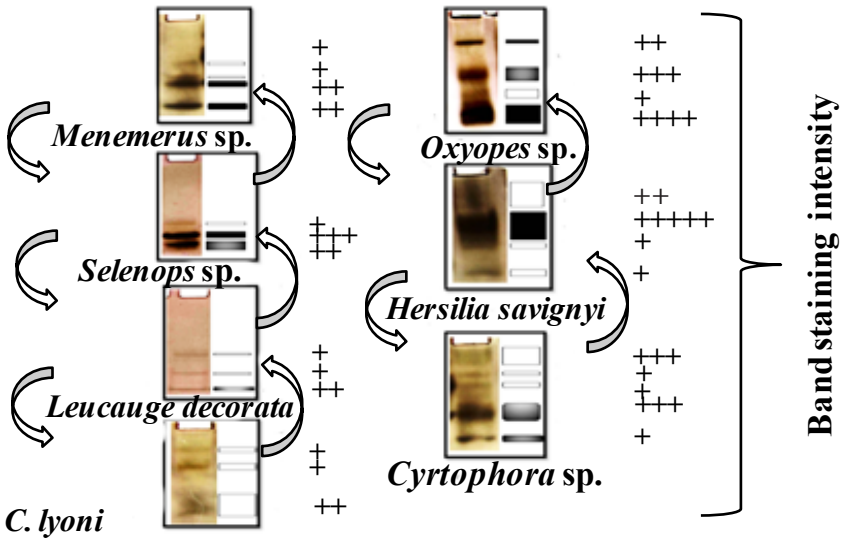


Figure 7- Non-specific esterase model for spiders

DISCUSSION

Morphological characterization and physiological differentiation are complement to each other and, therefore, often the appearance or disappearance of gene locus of single specific enzyme is considered as a good biochemical genetical marker, thereby, making it mandatory. Biochemical investigations for genetic diversity studies are essential to unravel the species specific patterns. Efforts in this regards towards spiders is very limited. The present study unraveled species specific profile of the seven families.

Alcohol dehydrogenase (ADH-E.C.1.1.1.1) has been very well studied in invertebrates, largely in insects, such as *Drosophila.* The present investigations indicate close similarity in the isozyme pattern of *Drosophila.* The total number of band phenotypes scored were three and the presence of three band isozymic phenotypes confirms the heterodimeric nature of the enzyme. These are designated as locus A and B and their heterodimer ADH-AB (Fig. 2). Presence of single locus with two alleles have been earlier reported by (McDonald and Avise, 1976) in *Drosophila.* This finding should be considered as a possible confirmation of mechanism of ADH isozyme expression in invertebrates at large. Following model have been projected for the basic expression of the different loci in the subjected species. The adults of *Menemerus* sp.and *Selenops* sp. showed low intense ADH-A locus as compaired to ADH-B zone.

The proportions of these electrophoretic loci expressions also may vary with developmental stages (Pasture and Kastritsis, 1971). Healthy insect which were under ethanol selection showed mainly the anodal band A, while the starved flies which were exposed to ketones exhibitted high fractions of cathodal form B (Johnson *et al.,* 1988). The spiders were collected directly from fields and were used for genetic analysis; this may be one of the reasons for the categorization and expression of the locus in species.

The model compared the species on the basis of the expression and intensity of the loci B and A. The species *Selenops* is placed at the top because it has all the possible genes present in the forms of loci A,B and AB. The intensity of the B locus increased in the evolution process so we placed *C. lyoni* in the derived ones (Fig.5).

ADH bands mentioned before are dependent upon the concentration of the substrate. Its activity indicate the effect of the vegetation and habitat from where they have been collected (Winberg *et al.,* 1982).There are possibilities about other factors which may be responsible for switching off and switching on the ADH gene at any locus. But ADH-B locus in spiders *C. lyoni*, *Leucauge decorata* and *Cyrtophora* sp. indicated more towards the food and habitat as the main factors. The comparative study of these different species thus provided evolutionary divergence between the spiders.

G6PD-Glucose-6-phosphate dehydrogenase (E.C.1.1.1.49) is considered as house keeping gene and is present in most tissues of multicellular organisms. This enzyme is X-linked so in the present study sex- specific expressions are established and genetic variations in G6PD is also observed. The females of *Cyrtophora* sp. and *C. lyoni* showed difference in number of bands and types of bands. G6PD-A and G6PD-B were expressed in females indicating the sex- differential nature of enzymes as observed (Smith and Engel, 1994). G6PD-B scored most conservative allele in all species of spiders. Model prepared for G6PD showed the dimeric X-linked phenomena where the *Selenops* sp. (♂) and *Cyrtophora* sp. ♀ were placed at the top as conservative one and *C.lyoni* is placed as derived one having intense A locus. G6PD can prove to be the significant tool to solve the sex determination and also help to probe the sex-reversal phenomenon occurring in these species.

Non-specific soluble esterase have attracted a great deal of attention as it displays extensive genetic polymorphism, but this issue is yet to be resolved whether these polymorphisms are maintained by natural selection or by any other factors. The esterases have many broad biological functions and they have extensively studied in insects and are involved in different physiological processes. The phenotype of non-specific soluble esterase in the seven species of spiders is reported for the first time from India.

The total number of electrophoretically distinct bands of esterases isozyme identified in the body tissue of spiders was three (Fig 4). They were numbered according to their rate of migration from the origin. Sub band hybrid formation was also visualized in seven spiders. The zone E1 follows the other E2 and E3 (Park and Kmable, 1999). The E3 zone was observed as highly cathodal while the E1 migration as anodal. There seems to be two clusters, one with presence of all these three functional zones found in four species (*Oxyopes* sp., *Hersilia savignyi*, *Cyrtophora* sp., *C. lyoni*) and the other cluster with E3 and E2 zones (*Menemerus* sp. , *Selenops* sp. and *Leucauge decorata*). In *Oxyopes* sp. there is high activity of E1 >E2>E3 zones. An additional band E3′ was also observed near E3 zone (Fig.7). These additional bands are the heterodimer of homodimer E2 and E3 as commonly found in insects.

Primitive spiders are homogenous and the recent once have high rate of heterozygosity in our study. The cause of the this phenomenon can be assigned to newly mutated and duplicated gene loci at early stages of allelic differentiation which yielded products which are structurally and metabolically similar allowing the formation of heterodimer. This study provides an evidence on the evolutionary status of spiders at allozymes level. The most variant group Pholcidae (*C. lyoni*) at the higher allelic variation is expected to be the phase of adaptation (Anjali and Prakash, 2014).

REFERENCES

Anjali and S. Prakash. 2012. Diversity of spiders from Semi-Arid habitat of Agra. Indian Journal of Arachnology 1(2):66-72.

Anjali and S. Prakash 2014. Cytogenetical investigations on spiders of semi-arid areas. Indian Journal of Arachnology, 3(2):40-54.

Edenberg, H. J. 2007. The Genetics of Alcohol Metabolism Role of Alcohol Dehydrogenase and Aldehyde Dehydrogenase Variants. Alcohol Research & Health Vol. 30, No. 1, 5-

Johnson, F. M., S.R. Bealle and G.R.B Webster. 1988. Isozyme variability in species of the genus *Drosophila* V Ejaculatory bulb esterases in the Drosophila Phylogeny. Biochem. Genet.2, 1.

McDonald, J. F. and J.C. Avise. 1976. Evidence for the adaptive significance of enzyme activity levels: interspecific variation in GPDH and ADH in *Drosophila*. Biochemical Genetics, Vol. 14, Nos. 3/4.

Park, N. J. and S.T. Kamble. 1999. Disstribution and inhibition of esterase in various body tissue of susceptible and resistant German cockroaches (Dictyoptera:Blattellidae). Entomologiacal society of America.V 92(4).556-562.

Pasture, N., and C.D. Kastritsis. 1971. Developmental studies in *Drosophila.*1.acid phosphatases, esterases and other proteins in organs and whole –fly homogenates during development of *D. pseudoobscura.* Dev. Biol., 26,525.

Prakash, S. 1990. Nich width allocation by Allozyme pattern. Microbes Letter 18:16-22.

Sambrook , J. and Maccallum. 2000. Molecular cloning :A laboratory Manual Third edition.

Smith, D. R. and M. S. Engel. 1994. Population structure in an Indian cooperative spider, *Stegodyphus sarasinorum* Karsch (Eresidae). Journal of Arachnology 22:108-113.

Winberg, J. O., D. R. Thatcher and J. S. Mckinley-Mckee. 1982. Alcohol dehydrogenase from the fruitfly *Drosophila melanogaster*. Inhibition studies of allozymes ADH. Biochem. Biophys. Acta. 704, 17-25.